

A complex B chromosome system in the Korean field mouse, *Apodemus peninsulae*

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Abstract. Information on B chromosomes of six subspecies of *A. peninsulae* Thomas, 1906, from 79 local populations of Russia (Siberia, Altai, Buryatia and the Far East), Mongolia, China, Korea and Japan (Hokkaido) is reviewed. The frequency of animals with B chromosomes is higher in this taxon than in other mammals and ranges from 0.4 up to 1.0, excluding two insular populations (Sakhalin Island and Stenin Island, Primorye) where Bs were not found. The B chromosome polymorphism shows four levels of variation in number (intraindividual

al mosaicism, intrapopulational and interpopulational), as well as variability in size, morphology and differential staining. Geographic variation was found among populations for these cytogenetic characteristics and, in some cases, it coincided with subspecies distribution. Comparative chromosome banding of micro and macro Bs illuminates possible pathways for their origin.

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Introduction

The Korean field mouse, *Apodemus peninsulae* Thomas, 1906 is a polytypic species which has been subdivided into nine (Corbet, 1978; Musser and Carleton, 1993) or six (Pavlenko, 1989) subspecies, dependent on the investigators. This species is widely distributed from East Siberia and North Mongolia, China to the Russian Far East, Korea and Japan (Hokkaido) (Fig. 1). Each subspecies occupies its own geographical region and has a slight differentiation in morphometric character (Vorontsov et al., 1977; Koh and Lee, 1994), the transferrin gene (Pavlenko, 1989), and mitochondrial DNA (Serizawa et

al., 2002). *A. peninsulae* belongs to the genus *Apodemus*, in which six species have been shown to carry B chromosomes (Kartavtseva, 2002; Wójcik et al., 2004). However, the Korean field mouse shows the widest spectrum of B chromosome variability and about 100% of animals with Bs are found in most populations. Many investigators have analysed B chromosome variation in size, morphology and number in local populations of *A. peninsulae* but, unfortunately, papers were often published in inaccessible journals or used different sets of information. All of these obstacles are among the causes determining that, in spite of numerous investigations on *A. peninsulae* B chromosomes, no comprehensive review is still available. In this paper we examine chromosomal data in 1,158 animals belonging to six subspecies of *A. peninsulae*, collected at 79 local populations from Russia (Siberia, Altai, Buryatia and the Far East), Mongolia, China, Korea and Japan (Hokkaido). Data gathered from our own studies, and from the literature, are considered (Table 1, Fig. 1). Table 1 summarizes the B chromosome data, including their number, morphology, type of differential staining and number of mosaics in each examined locality. We also calculated the frequency of animals with macro and micro B chromosomes for geographical regions using our own information and the literature (Table 2).

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Fig. 1. Geographic distribution of *A. peninsulae* Thomas and collection numbers. Arabic numbers beside points are location numbers shown in Table 1. Solid dots show populations with B chromosomes, and squares show populations lacking Bs.

Table 1. Karyotypic characteristics in the Korean field mouse, *Apodemus peninsulae* Thomas, from different localities

Locality ^a	No. of animals ^b		No. of B chromosomes ^b			Type of differential staining ^b	References
	Total	Mosaics	Total	Macro	Micro		
<i>A. p. nigritalis</i> Hollister, 1913							
Siberia, Russia:							
1. Tomsk and Tomsky Region	32	10	0–13	0–7	0–6	G, C	Kral, 1971; Volobuev, 1979, 1980a; Bekasova et al., 1980; Timina et al., 1980; Roslik et al., 2003
2. Novosibirsk	9	2	2–12	2–7	0–5	C, FISH	Kral, 1971; Radjabli and Borisov, 1979; Borisov, 1990a; Karamysheva et al., 2002; Trifonov et al., 2002
3. Altai, Cherga, Shebalinskiy District	6	6	4–11	0–6	2–8	C, FISH	Our data; Trifonov et al., 2002
4. Teletskoye Lake	51	12	0–7	0–7	0	C, NOR	Kral, 1971; Borisov, 1990a; Borbiev, 1991
5. Novoangarsk, 6. Kan River and 7. Krasnoyarsk	50	17	2–18	1–10	0–14	C	Borisov, 1990c; Radjabli and Borisov, 1979; Kolomiets et al., 1988
8. Yenisei River (left shore)	39	13	1–8	0–6	0–5	G, NOR	Borisov, 1986, 1990c; Borbiev, 1991
9. Yenisei River (right shore) and 10. Sayanogorsk	39	15	1–12	0–5	0–10	C, G, NOR	Borisov, 1986, 1990b, 1990c; Borbiev, 1991
11. Maina	9	9	13–24	1–4	12–20	–	Volobuev, 1979, 1980a; Timina et al., 1980
12. Kizil, Tyva	2	2	13–18	0–1	13–17	C	Roslik et al., 2003; our data
13. Valley of Ubsunur Lake, Tuva	5	5	2–8	1–5	0–3	–	Roslik et al., 2003; our data
Spermatocytes**							
Animals from locality 4	2	–	0–3	0–3	0	–	Ishak et al., 1991
Animals from localities 4 and 7	11	11	1–12	–	–	–	Kolomiets et al., 1988; Borbiev et al., 1990
Animals from localities 1 and 11	3	–	6–20	–	–	–	Popova et al., 1980
Subtotal	242	91	0–24	0–10	0–20	C, G, NOR, FISH	
Baikal Lake region***:							
14. Barda and 15. Kochergat	56	0	1–7	1–3	0	–	Borisov, 1990d; Zima and Macholan, 1995
16. Baikalsk and 17. Babushkin	36	22	5–14	0–5	3–14	–	Borisov, 1990d; Borbiev, 1991
Spermatocytes**							
Animals from locality 16	2	–	1–3	–	–	–	Ishak et al., 1991
Subtotal	92	22	1–14	0–5	0–14	–	

Table 1 (continued)

Locality ^a	No. of animals ^b		No. of B chromosomes ^b			Type of differential staining ^b	References
	Total	Mosaics	Total	Macro	Micro		
Buryatia:							
18. Ivolginsk, 19. Gusinoozersk and 20. Ulan-Ude	9	4	2-4	0-2	1-4	G, NOR	Borisov, 1990d; Borisov and Malygin, 1991; Borbiev, 1991
Chita Region:							
21. Novokruchininskiy	3	1	1-5	1-4	0-1	-	Roslik et al., 2003
22. Sretensk and 23. Boti	6	6	3-9	0-3	2-9	-	Kartavtseva, 2002; Roslik et al., 2003
Subtotal	18	11	1-9	0-4	0-9	G, NOR	
<i>A. p. major</i> Radde, 1862 non Pallas, 1779							
Mongolia:							
NE Mongolia***	12	-	1-7	-	-	-	Zima and Macholan, 1995
N Mongolia***	1	1	2-4	1	2-3	-	Kolomiets et al., 1988
Spermatocytes**	1	1	1-7	-	-	-	Kolomiets et al., 1988
24. Hangay, East Hantey	4	0	2-7	1-5	0-2	-	Borisov and Malygin, 1991
25. Bulgansky Aymak and 26. Monastery Amar-Hiid	21	4	2-13	1-3	1-11	-	Borisov and Malygin, 1991
27. Foothills Great Hingan	1	0	1	1	0	-	Borisov and Malygin, 1991
Subtotal	39	5	1-13	1-5	0-11	-	
<i>A. p. praetor</i> Miller, 1914							
Far East, Russia: 28. Magadan,							
Amursky Region: 29. Belogoriye,							
Jewish Autonomous Region: 30. Birakan and							
Khabarovsk Region:							
31. Krasnoye	20	11	0-4	0-4	0	-	Kartavtseva et al., 2000; Roslik et al., 2003; our data
32. Evoron Lake, 33. Komsomolsk-na-Amure and 34. Mariinskoye	29	22	0-7	0-7	0-1	FISH	Rubtsov et al., 2004, this volume; our data
35. Malyshevo, 36. Pivan and 37. Sovetskaya Gavan	19	12	0-4	0-4	0	-	Kartavtseva et al., 2000; Roslik et al., 2003
Subtotal	68	45	0-7	0-7	0-1	FISH	
Primorsky Region***:							
38. Krasny Yar, 39. Melnichnoye and 40. Sikhote-Alin Reserve	9	9	0-4	0-4	0	-	Bekasova and Vorontsov, 1975; Bekasova et al., 1980; Bekasova, 1984; Borisov, 1990a; Kartavtseva et al., 2000
41. Rudnaya Pristan	14	9	0-6	0-6	0-3	G	Kartavtseva et al., 2000
42. Dalnegorsk, 43. Khrustalny and 44. Olga	45	33	0-5	0-5	0	C, G	Roslik et al., 2003
45. Chuguevka and 46. Busseyevka	5	5	0-5	0-5	0-1	-	Kartavtseva et al., 2000
47. Arsenievka River and Nikolaevka	8	6	0-3	0-3	0	-	Kartavtseva et al., 2000; Roslik et al., 2003
48. Ussuriysky Reserve	103	47	1-5	0-5	0-3	C, G, FISH	Bekasova and Vorontsov, 1975; Kartavtseva et al., 2000; Trifonov et al., 2002
49. Novonezhino (3 sample sites)	13	10	0-5	0-5	0-2	-	Kartavtseva et al., 2000; Roslik et al., 2003
50. Novolitovsk* and 51. Avangard	14	9	1-4	1-4	0	C, G	Kartavtseva et al., 2000
52. Vladivostok	8	1	1-4	1-4	0-2	-	Kartavtseva et al., 2000
53. Turiy Rog (2 sample sites) and 54. Pogranichny (2 sample sites)	6	4	0-4	0-4	0	-	Kartavtseva et al., 2000
55. Granitnaja River and 56. Nezhino	16	11	0-7	0-5	0-3	-	Kartavtseva et al., 2000
57. Kedrovaya Pad Reserve	67	48	0-5	0-5	0-1	C, G, NOR	Kartavtseva et al., 2000
58. Ryazanovka, 59. Kraskino and 60. Khasan	9	3	1-4	1-4	0	-	Kartavtseva et al., 2000
61. Gamov peninsula	6	6	0-5	0-5	0-1	-	Kartavtseva et al., 2000
62. Russky Island	13	11	0-5	0-4	0-2	-	Kartavtseva et al., 2000
63. Stenin Island	16	0	0	0	0	C	Roslik et al., 2003
Subtotal	464	255	0-7	0-6	0-3	C, G, NOR, FISH	
Laboratory population (from locality 48)	10	9	0-6	0-4	0-4	-	Boeskorov et al., 1995; Roslik et al., 1998
<i>A. p. peninsulae</i> Thomas, 1906							
Far East, Korea:							
64. Mungyong, 65. Kwangnyung, Kyunggido and 66. Gyeonggi Do	21	1	1-6	1-6	0	C, G, Q	Koh, 1986; Abe et al., 1997; Sawaguchi et al., 1998
<i>A. p. giliacus</i> Thomas, 1907							
Far East, Russia, Sakhalin Island:							
67. Okha, 68. Aleksandrovsk, 69. Tymovsk, 70. Sokol, 71. Tomari and 72. Gornozavodsk	88	0	0	0	0	C, G, Q	Bekasova, 1984; Zima and Macholan, 1995; Kartavtseva et al., 2000; Roslik et al., 2003
Tail fibroblast culture (from locality 67)**	7	-	+	+	+	C, G, Q	Sawaguchi et al., 1998

➤

Table 1 (continued)

Locality ^a	No. of animals ^b		No. of B chromosomes ^b			Type of differential staining ^b	References
	Total	Mosaics	Total	Macro	Micro		
Far East, Japan, Hokkaido Island:							
73. Naganuma	27	–	3–13	1–5	1–9	Q	Hayata et al., 1970; Hayata, 1973
Litters from pregnant wild females	28	–	2–9	1–5	1–6	–	Hayata, 1973
74. Hitujigaoka, Sapporo and 75. Hayakita	5	0	2–6	0–4	2	C, G, Q	Abe et al., 1997
76. Tomakomai	7	6	4–10	0–4	4–9	C	Roslik et al., 2003
Subtotal	67	7	0–13	0–5	0–9	C, G, Q	
Laboratory population (from location 73)	31	–	0–6	0–3	0–6	–	
Fibroblast cultures (1 female + fetuses)**	7	–	2–10	–	–	–	Hayata, 1973
Male: Bone marrow**	1	1	5–6	–	–	–	Hayata, 1973
Spermatogonia**	1	0	6	–	–	–	Hayata, 1973
Lung (culture)**	1	1	6	–	–	–	Hayata, 1973
Heart (culture)**	1	1	5–6	–	–	–	Hayata, 1973
Culture	1	1	5–6	–	–	–	
<i>A. p. praetor</i> Miller, 1914							
China:							
77. Mt. Changbai, Jilin Prov.	5	2	0–3	1–3	0	C, G, NOR	Wang et al., 2000
<i>A. p. sowerbyi</i> Jones, 1956							
78. Mt. Tai, Shandong Prov.	7	5	5–14	1	4–13	C, G, NOR	Wang et al., 2000
79. Mt. Qinling, Shaanxi Prov.	5	2	0–1	0–1	0	C, G, NOR	Wang et al., 2000
Total	1158	455	0–24	0–10	0–20	C, G, NOR, FISH	

^a *: Macro B chromosome. **: Animals that were not counted in the total sum because they had already been registered in their locality. ***: Without numbers; authors did not provide specific sampling sites.

^b +: Number of chromosomes is unknown. –: No data.

B chromosome morphology

B chromosomes, in addition to the standard diploid set of *A. peninsulae*, were described in a taxon named “*A. giliacus*” (Hayata et al., 1970) in the Hokkaido population and in a taxon named “*A. speciosus*” (Kral, 1971) in the Siberia population. The standard (A) diploid set of *A. peninsulae* contains 48 acrocentric chromosomes gradually decreasing in size. In addition, up to 24 supernumerary (B) chromosomes may be found in some individuals. In this paper, we divide B chromosomes into two groups according to their size. The first group includes Bs of visible morphology under light microscopy (macro Bs), which are larger or equal in size to the smallest A chromosome (Fig. 2A, C, D). The second group includes only dot-like chromosomes which are much smaller than A chromosomes and without clear morphology (micro Bs) (Fig. 2B). The macro B chromosomes are divided into classification types according to their morphology and relative size in comparison with the A chromosomes. We have previously described three types of macro Bs (Kartavtseva et al., 2000), with abbreviations determined by reference to some other sources (Hayata, 1973; Borisov, 1986; Abe et al., 1997): (1) large metacentrics or submetacentrics (Lm-sm); (2) medium-to-small metacentrics or submetacentrics (Mm-sm); (3) large-to-small acrocentrics or subtelocentrics (A-St). The most frequent macro Bs in *A. peninsulae* are Mm-sm (Fig. 2A), whereas A-St morphological variants of medium or small size are rare. An extremely large St chromosome, which was significantly larger than the largest A

chromosome, was found in one male from locality no. 50 (Fig. 2D) (Kartavtseva et al., 2000). In some cases we could identify the morphology of micro Bs in good quality metaphase plates, but, in most cases, micro Bs looked like very small structures without clear morphology.

B chromosome number

The number of B chromosomes in continental populations of *A. peninsulae* varies from 0 to 24 (Table 1), although this extreme value was registered only once, in a population from Siberia (locality no. 11). The number of macro B chromosomes varies from one to ten in some Siberian localities, and from one to three in some localities from China. Geographical and interpopulational variation in the number and morphology of macro Bs is insignificant (Kartavtseva, 2002). In contrast, a high variation in the number of micro Bs is the rule, with a broad variation at inter- and intrapopulation levels: 0–20 in the southern area of Siberia, 0–11, in Siberia and Mongolia (*A. p. nigritalus* and *A. p. major*), 4–13 in China (*A. p. sowerbyi*), 1–9 in Hokkaido (*A. p. giliacus*) and 0–3 in the Far East (*A. p. praetor*) (Table 1).

B chromosome frequency

The frequency of animals carrying B chromosomes (B-prevalence) varies among localities and regions, although most populations analysed show a high B-prevalence, with most individuals carrying B chromosomes of one type or another (Table 2).

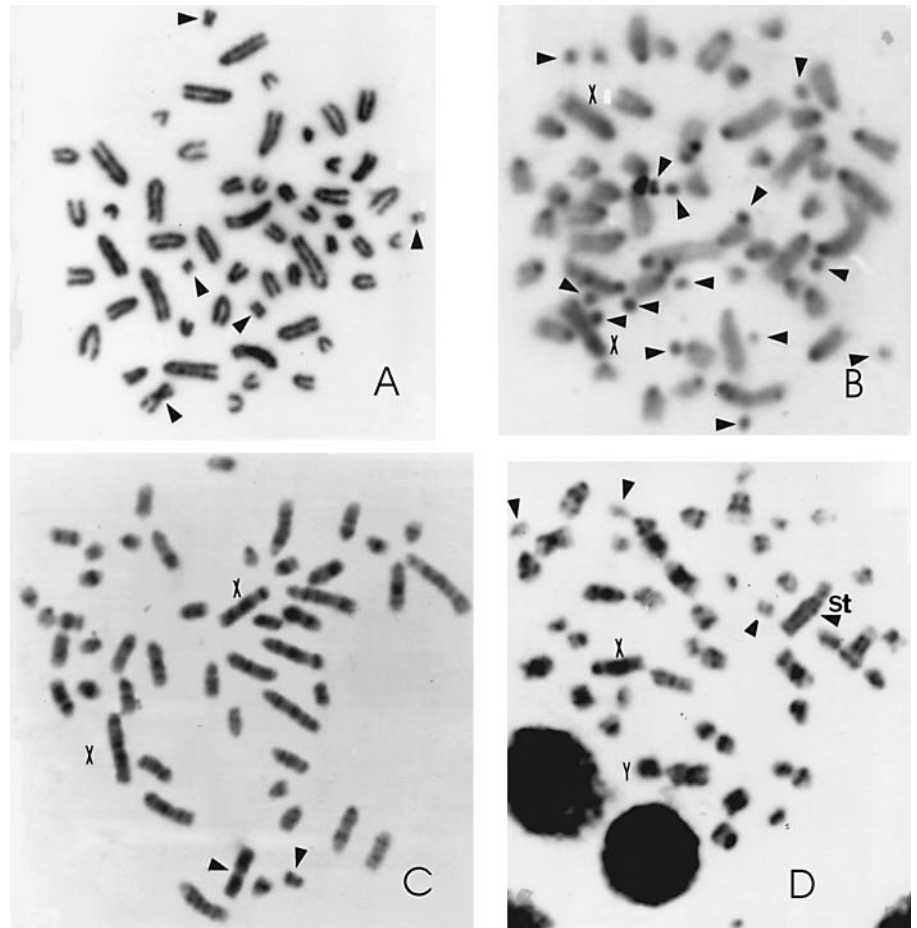


Fig. 2. Metaphase plates of *A. peninsulae* with different B chromosome morphology and size. **(A)** Cell showing Mm-sm and micro B chromosomes (locality no. 13); **(B)** C-banded cell showing micro B chromosomes (locality no. 12); **(C)** G-banded cell showing Lm-sm and Mm-sm (locality 48); **(D)** G-banded cell showing large St and Mm-sm B chromosomes (locality no. 50). Arrows indicate B chromosomes. Sex chromosomes are marked with X and Y.

Populations lacking B chromosomes (prevalence = 0) were found in two islands, Sakhalin and Stenin. In Sakhalin, which is a large island, none of the 88 individuals hitherto analysed carried B chromosomes. In Stenin, a very small island, we analysed the karyotypes of mice over a two-year period but did not find any B chromosome in a total sample of 16 individuals (Roslik et al., 2003). At the other extreme, for instance, only two animals lacked B chromosomes in Siberia (*A. p. nigritalus*) (localities nos. 1 and 4) and B prevalence in this region was about 0.99. In addition, all animals from Korea (*A. p. peninsulae*) carried B chromosomes (prevalence = 1). In two Chinese populations (*A. p. sowerbyi*), however, B prevalence was very different (1 and 0.4).

Almost all B carrying individuals in this species harbour macro B chromosomes. The frequency of animals carrying macro Bs shows only slight differences among regions or subspecies (Table 2).

Micro B prevalence broadly differs among regions and subspecies (Table 2). Four subspecies, *A. p. nigritalus* from Siberia, Buryatiya and Chita Region, *A. p. major* from Mongolia, *A. p. giliacus* from Hokkaido and *A. p. sowerbyi* from China, show a very high frequency of animals with micro Bs (prevalence from 0.59 to 1). On the contrary, two subspecies from the Far East (*A. p. peninsulae* and *A. p. praetor*) and one from the Baikal Lake Region (*A. p. nigritalus*) showed lower values of micro B

prevalence (0–0.39). In three localities from Siberia, nos. 4, 14 and 15, micro Bs were not found even though they are frequent in other localities in this region (see Table 1). In wild populations from the Far East, micro Bs are scarce or absent (they were found in only 46 out of 532 animals analysed). Micro B chromosomes commonly occurred together with bi-armed B chromosomes. However, some animals from Siberia (locality nos. 8 and 13), Chita Region (localities nos. 22 and 23), Buryatia (locality no. 19), Mongolia (locality no. 25), Hokkaido (locality no. 73) and the Russian Far East (laboratory population from locality no. 48) carried only micro B chromosomes.

Mosaicism for B chromosomes

Intra-individual variation for B number in somatic tissues has been reported in the Korean field mouse (Bekasova and Vorontsov, 1975; Radjabli and Borisov, 1979; Volobuev, 1980a). In addition, mosaicism for B morphology in animals with a stable number of B chromosomes has also been reported (Bekasova et al., 1980; Borisov, 1980, 1990c). Mosaicism of one kind or another is a frequent characteristic of B chromosomes in this species (see Table 2).

In a previous study, mosaicism was estimated from the presence of two or more cellular clones within a same individual (Belyaev et al., 1974). A cellular clone is a group of cells with a definite diploid number. In cases where the proportion of hypo-

Table 2. B chromosome characteristics in the Korean field mouse, *Apodemus peninsulae* Thomas from different regions

Region	Number of animals	Frequency ^a of animals with				Response of macro B chromosomes ^b to	
		Bs	macro Bs	micro Bs	mosaicism	G-banding	C-banding
<i>A. p. nigritalus</i>							
Russia: Siberia	242	0.99	0.96	0.59	0.37	G-bands	C-bands, some negative (like Y chromosome)
Baikal Lake Region	92	1.0	0.98	0.39	0.24	–	–
Buryatia	9	1.0	0.89	1.0	0.44	G-bands	–
Chita Region	9	1.0	1.0	1.0	0.78	–	–
<i>A. p. major</i>							
Mongolia	39	1.0	0.97	0.85	0.13	–	–
<i>A. p. praetor</i>							
Russian Far East:Magadan, Amursky Region	8	1.0	1.0	0	0.63	–	–
Khabarovsk Region	60	0.78	0.78	0.07	0.67	–	–
Primorsky Region	445	0.9	0.89	0.11	0.57	Negative	Negative
China (locality no. 77)	5	1.0	1.0	0	0.4	Negative	Negative
Islands: Russia: Russky Stenin	13	1.0	1.0	0.23	0.85	–	–
	16	0	0	0	0	–	–
<i>A. p. giliacus</i>							
Sakhalin	88	0*	0	0	0	–	–
Japan: Hokkaido	98	1.0	0.91	0.97	0.85**	Dark or partially banded	Negative but centromeric C-bands
<i>A. p. peninsulae</i>							
Korea	21	1.0	1.0	0	0.05	Negative	Negative
<i>A. p. sowerbyi</i>							
China (locality no. 78)	7	1.0	1.0	1.0	0.71	Negative	Negative
(locality no. 79)	5	0.4	0.4	0	0.4	Negative	Negative

^a *: The B chromosomes (macro and micro) arose de novo after polyploidization in the tail culture system. ** Only valid for locality 76.

^b –: Not done.

ploid cells is higher than 10%, and that of hyperploid cells is 5% or more, as deduced from the modal number of chromosomes, we considered these cells as cellular clones (Belyaev et al., 1974).

The main types of B chromosomes in this species are bi-armed and thus they are easily distinguishable from acrocentric A chromosomes. To calculate the 2n number, we separately counted A and B chromosomes and did not find any mosaicism affecting A chromosomes.

The frequency of animals with mosaicism varied among subspecies. The highest proportion of mosaic individuals was found in *A. p. praetor* and *A. p. giliacus* (0.85), *A. p. sowerbyi* (0.71), *A. p. nigritalus* (0.78). The lowest values were found in *A. p. major* (0.13), and *A. p. peninsulae* (0.05) (Table 2).

For the two Primorsky populations (localities nos. 48 and 57), seasonal variation in the frequency of mosaics was observed in association with population size (Kartavtseva, 1999). Data derived from the literature on chromosome numbers in *A. peninsulae* allow the assumption that there will be a similar correlation in the populations of Siberia (Kartavtseva, 2002).

Banding of B chromosomes

The G-banding patterns of all macro B chromosomes in mice from Siberia (*A. p. nigritalus*) and some of the macro Bs found in mice from Hokkaido (*A. p. giliacus*) are similar to those of some A chromosomes. On the other hand, macro Bs in mice from the Far East (*A. p. praetor*, *A. p. giliacus*, *A. p. penin-*

sulae) and China (*A. p. sowerbyi*) show no G-bands (Tables 1 and 2, Fig. 2 C and D).

C-banding response of macro B chromosomes revealed three different patterns: (1) centromeric and interstitial darkly stained bands, found in *A. p. nigritalus* populations from West Siberia (Radjabli and Borisov, 1979; Borisov, 1990a; Trifonov et al., 2002); (2) only centromeric darkly stained bands, found in *A. p. giliacus* from Hokkaido (Abe et al., 1997) and Sakhalin (tissues cultures, Sawaguchi et al., 1998); and (3) C-negative or gray stained bands, or poorly stained heterochromatin, found in *A. p. praetor* Bs from the Russian Far East (Bekasova et al., 1980; Kartavtseva et al., 2000; Kartavtseva, 2002), in *A. p. peninsulae* Bs from Korea (Abe et al., 1997), China (Wang et al., 2000) and in *A. p. sowerbyi* Bs from China (Wang et al., 2000) (Table 2). This variation in C-banding response suggests that macro Bs from different subspecies or regions contain a heterogeneous collection of heterochromatic genetic material (Kartavtseva, 2002).

Micro B chromosomes in mice from all investigated localities usually show G-negative but C-positive staining (Fig. 2B). The micro Bs from the Hokkaido population were reported to be completely negative for QM staining but fluoresced brightly after CMA₃ staining (Sawaguchi et al., 1998), suggesting that they contain GC-rich C-heterochromatin, at least in this population.

In one *A. p. praetor* specimen from the Primorsky Region, nucleolus organizer regions (NORs) were observed on its two

macro B chromosomes (Mm-sm) (Boeskorov et al., 1995), suggesting that Bs might carry rRNA genes. However, in many other animals investigated from this and other populations, NOR-banding on both macro and micro Bs was not found (Borbiev, 1991; Wang et al., 2000; Kartavtseva, 2002). It seems that the presence of rRNA genes is also heterogeneous among Bs. Based on B chromosome morphology and G-banding, it was suggested that many B chromosomes in *A. p. nigritalus* are isochromosomes (Kolomiets et al., 1988; Borbiev, 1991), which was later confirmed by FISH (Karamysheva et al., 2002). This technique has also show that macro and micro Bs contain two types of B-specific heterochromatin (Trifonov et al., 2002) and large amounts of repeated DNA sequences. DNA repeats, obtained from B chromosomes by microdissection, located on pericentromeric regions of B chromosomes, were also present in the pericentromeric C-blocks of all autosomes and in non-centromeric C-blocks of sex chromosomes (Karamysheva et al., 2002).

Interesting results were obtained in tissue cultures (Sawaguchi et al., 1998). The modal number of chromosomes in cultured cells from heart, lung and skin tissues was, as a rule, constant, but three out of seven specimens from the Sakhalin Island (locality no. 67, where Bs were never found) showed growing fibroblasts with polyploid cells and with Bs even in the primary cultures. The observed Mm-sm and micro B chromosomes presumably appeared de novo and became visible after polyploidization in the tail culture. Bi-armed Bs contained GC-rich heterochromatin in the centromere regions and AT-rich heterochromatin in the arm regions, but micro Bs were completely GC-rich (Sawaguchi et al., 1998).

B chromosomes at meiosis

Meiosis behavior of B chromosomes is, as a rule, irregular. The micro B chromosomes do not show pairing at metaphase I, remaining as univalent, whereas other B chromosome types show univalents and, rarely, bivalents (Wang et al., 2000) and multivalents (Hayata, 1973; Popova et al., 1980; Kolomiets et al., 1988; Ishak et al., 1991; Borbiev et al., 1990). An increase in the number of B chromosomes was found in zygotene-pachytene spermatocytes relative to that found in bone marrow cells, which may be evidence of B chromosome accumulation in the germ cell line of mice from Siberia (locality no. 7) and North Mongolia (Kolomiets et al., 1988). B chromosomes from Siberian mice showed associations with the X and Y chromosomes at pachytene (Borbiev et al., 1990).

Laboratory populations

Laboratory populations of Korean field mice from two local populations have been studied (Table 1). The results of breeding experiments and karyotype analysis with four parental pairs of *A. peninsulae* from Hokkaido (locality no. 73) and their twenty-six offspring showed the occurrence of B variants in the offspring which were not present in the mother (Hayata, 1973). In the continental population no. 48, the experimental tick-born encephalitis (TBE) virus was reproduced in a pregnant female by inoculation with an excessive amount of TBE virus. Ten offspring were obtained from this infected female and her progeny following an inbreeding program (up to F6). Karyo-

types of the mice yielded by this female were analyzed, but the karyotype of the infected female was unknown. In addition to the 48 A chromosomes, 1 to 6 Bs were found, including 0–4 Mm-sm and 0–4 micro Bs. Four of the ten mice only carried micro Bs. The micro Bs are usually rare in mice from this locality and their number did not exceed 3 in the natural population. While B prevalence was only 0.11 in specimens from the Ussuriysky Reserve wild population, it was 1 in laboratory populations (our data). A hypothesis has been suggested that inoculation of artificial TBE may cause some chromosomal alterations which thus increase the frequency of micro Bs in *A. peninsulae* (Roslik et al., 1998; Kartavtseva, 1999). The absence of Bs in chromosomal sets of *A. peninsulae* from Sakhalin had been formerly anticipated to be due to TBE absence in mice populations from this island (Bekasova and Vorontsov, 1975; Bekasova, 1984).

The origin of B chromosomes

There are several points of view about the origin of B chromosomes in mammals (Volobuev, 1978, 1980b, 1981). In *A. peninsulae* it is possible to consider several ways of B chromosome origin, perhaps taking place simultaneously. The similarity of G- and C-banding among the A and B chromosomes in this species from the Siberian population support the origin of Bs from the A chromosomes, which is also indicated by the association of Bs with sex chromosomes at pachytene (Kartavtseva, 2002).

We found high numbers of micro Bs in a population near a lead factory (locality no. 41), which suggested that lead pollution might favour a B frequency increase in this population, but we did not find micro Bs in a population near a polymetal factory (locality no. 42) (Yakimenko et al., 1994). Therefore, the relationship between B frequency and environmental pollution by heavy metals is not substantiated properly. B frequency is distinctively high in many Siberian populations situated in areas that are apparently not affected by this kind of pollution. Furthermore, there are studies in another *Apodemus* species (e.g. *A. flavicollis*) which did not support such correlation (Zima et al., 1999).

The geographical heterogeneity of B chromosomes in *A. peninsulae* is consistent with spatial and temporal patterns of B chromosome variation observed in other B chromosome systems (Camacho et al., 2000). However, many important questions on B structure, molecular composition, effects and inheritance, remain to be answered to understand the reasons for B chromosome occurrence in *A. peninsulae*. The B chromosome system in this species is thus a good model for analysing the evolution of these enigmatic components of many eukaryote genetic systems.

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